Recent advances in understanding plant nuclear envelope proteins involved in nuclear morphology.

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REVIEW PAPER

Recent advances in understanding plant nuclear envelope proteins involved in nuclear morphology

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Abbreviations: INM, inner nuclear membrane, NE, nuclear envelope, Nup, nucleoporin; NPC, nuclear pore complex, ONM, outer nuclear membrane

A short statement: Plants have evolved unique machineries for regulating nuclear structure. In this review, we summarise plant nuclear envelope proteins that tightly control nuclear morphology.

Abstract

The nuclear envelope (NE) is a fundamental structure of the nucleus and plays an important role in nuclear morphology through the strict regulation of NE protein function. Beyond its physical barrier function between nucleoplasm and cytoplasm, recent studies of the plant NE have provided novel insights into basic aspects of nuclear morphology as well as cellular organisation. In this review, we focus on plant NE proteins that have emerged from recent studies in nuclear morphology, and we discuss their physiological functions in cellular activities. A better understanding of the NE protein functions should provide key insights into the physiological significance of proper nuclear structure in plants.

Keywords: lamina structure, LINC complex, nuclear envelope, nuclear morphology, nuclear pore complex, nucleoporin

Introduction

In eukaryotes, the nuclear envelope (NE) separates the nucleus and cytoplasm, providing a specialized microenvironment within the nucleus. The constituents of the NE are the outer nuclear membrane (ONM), the inner nuclear membrane (INM), the nuclear pore complex (NPC), and the nuclear lamina. The ONM is sometimes continuous with the endoplasmic reticulum (ER), but also contains ONM-specific protein complexes (Lusk *et al.*, 2007; Tzur *et al.*, 2006). Although the INM has a contiguous border with the ONM at the periphery of each NPC, it contains its own distinctive array of integral membrane proteins (Schirmer *et al.*, 2003). In animal cells, the composition of integral INM proteins may vary significantly between different cell types (Lusk *et al.*, 2007), suggesting that each INM protein has a specific function. In contrast, in plants, only a few INM homologues and no ONM homologues have been identified and, therefore, composition of the nuclear membrane in plants is largely unknown. Such low sequence similarity between animal and plant NE proteins indicates that plants have evolved unique machineries on the NE to perform nuclear processes. Recently, high resolution scanning electron microscopy (feSEM, an in-lens field emission scanning electron microscopy) has revealed detailed structure of the NE in cultured tobacco cells (Fiserova and Goldberg, 2010; Fiserova *et al.*, 2009). Fiserova *et al*. clearly demonstrated that structural organisation of the NPC and lamina structure of tobacco is very similar to those of vertebrates (Fiserova *et al.*, 2009). These results suggest that plants and animals share a fundamental NE structure.

NE research has been spurred on by the link between alterations in NE proteins and a variety of human diseases (Dauer and Worman, 2009; Jevtic *et al.*, 2014). Indeed, since the association between abnormal nuclear structure and tumour grade was discovered, nuclear morphology has been an indispensible criterion for pathological assessment of cancer (Chow *et al.*, 2012). In addition, it is known that

alterations to NE structure are essential to many aspects of metazoan development and cellular differentiation (Dauer and Worman, 2009). In *Arabidopsis*, nuclei exhibit cell-type specific morphology and undergo polymorphic morphological changes during long-distance movement (Chytilova *et al.*, 1999; Chytilova *et al.*, 2000; Tamura *et al.*, 2013). Such a striking and distinctive diversity in nuclear morphology suggests that plants have mechanisms that dynamically control the structure and protein constituents of the NE. Elucidation of NE structure dynamics is likely to lead to a better understanding of how plants regulate cellular activity and differentiation in response to environmental signals. Therefore, in this review, we highlight recent advances in the understanding of the plant NE proteins that regulate nuclear morphology and activity.

Plant nuclear lamina-like structure

The nuclear lamina is a filamentous structure located beneath the inner nuclear membrane and plays a critical role not only in NE organisation but also in various nuclear functions, including gene transcription and DNA replication (Burke and Stewart, 2013; Simon and Wilson, 2011). In animals, long coiled-coil proteins called lamins are major components of the lamina structure. Lamins are type V intermediate filaments and are dynamically assembled and disassembled into the network polymer during the cell cycle. Mutations in lamins or lamin-binding proteins cause severe inherited diseases, collectively termed laminopathies (Gruenbaum *et al.*, 2005; Zwerger and Medalia, 2013). It has been difficult to explore the molecular constituents of the plant lamina-like structure, because homologues of vertebrate lamin have not been found in plant genomes (Ciska and Moreno Diaz de la Espina, 2013, 2014). Recently several proteins have been proposed as candidates for the structural component of the plant lamina-like structure.

Nuclear matrix constituent protein (NMCP)/Crowded nuclei (CRWN)

Nuclear matrix constituent protein 1 (NMCP1) was biochemically isolated from nuclear matrix proteins in carrot (Masuda *et al.*, 1997). Immunogold labelling with anti-NMCP1 antibody demonstrated that NMCP1 are localised exclusively in an electron-dense layer at the nuclear periphery. Although there is no amino acid sequence similarity between animal lamin and plant NMCPs, they share similar secondary structure, including a non-coiled head, a central coiled-coil rod domain, and a tail domain (Ciska *et al.*, 2013). Phylogenetic analysis roughly classifies NMCPs into two subfamilies, NMCP1 and NMCP2 (Ciska *et al.*, 2013). Monocots have a single copy of each of NMCP, while dicots have multiple NMCP1s and a single NMCP2 (Ciska and Moreno Diaz de la Espina, 2014). However, the functional redundancy and diversity of NMCP1 and NMCP2 are not fully characterised.

Loss of function mutants of *Arabidopsis* NMCP homologues were isolated as *crowded nuclei* (*crwn*) mutants [originally named *little nuclei* (*linc*)] (Dittmer *et al.*, 2007). The nuclei in *crwn* mutants exhibit smaller and more spherical morphology compared to wild-type nuclei. *Arabidopsis* has four *CRWN* genes (*CRWN1-4*). CWRN1-3 belong to the NMCP1 subfamily, while CRWN4 belongs to the NMCP2 subfamily (Ciska and Moreno Diaz de la Espina, 2013, 2014; Wang *et al.*, 2013). Knockout mutant analyses reveal that CRWN1 and CRWN4 play predominant roles in nuclear morphology (Sakamoto and Takagi, 2013; Wang *et al.*, 2013), suggesting that NMCP1 and NMCP2 subfamilies have non-overlapping functions in the maintenance of nuclear morphology. Goto *et al*. also independently isolated a novel *crwn1* allele with a point mutation (referred to as a *kaku2* mutant) by forward genetics (Goto *et al.*, 2014). Over-expression of *CRWN1* leads to moderate nuclear membrane deformation in tobacco cells. Such a nuclear membrane deformation was observed in HeLa cells over-expressing lamin A and lamin B1 (Volkova *et al.*, 2011). Therefore, the total amount of CRWNs should be strictly controlled to form appropriate NE structures. In addition to nuclear morphology, chromocentre organisation is also altered in *crwn*

mutants (Wang *et al.*, 2013). *crwn4* exhibits mispositioning of the 5S RNA and centromeric repeats outside of chromocentres, indicating that CRWN4 is required for maintenance of higher order heterochromatin organisation in plants. Given that chromocentres are mainly localised on the nuclear periphery (Fransz *et al.*, 2002; Wang *et al.*, 2013) where CRWNs are also localised, CRWNs are considered important factors for heterochromatin organisation.

Sakamoto and Takagi thoroughly investigated the subcellular localisation of each CRWN with fluorescent protein fusions (Sakamoto and Takagi, 2013). In interphase cells of *Arabidopsis* leaf and root, CRWN1 and CRWN4 predominantly are localised at the nuclear periphery, while CRWN2 is localised in the nucleoplasm and CRWN3 is localised to the nuclear periphery and nucleoplasm. After NE break down in mitotic cells, CRWN2-4 disperse into the cytoplasm, whereas CRWN1 is found on the condensing chromatin. These results suggest that each CRWN has a different localisation and function. Interestingly, single knockout of each CRWN gene results in a normal growth phenotype similar to wild-type plants (Dittmer *et al.*, 2007; Sakamoto and Takagi, 2013; Wang *et al.*, 2013). However, some multiple knockout mutants show a dwarfed phenotype. Triple knockout mutants (*crwn1 crwn2 crwn4* and *crwn1 crwn3 crwn4*) exhibit extremely stunted growth and low fertility, while it has not been possible to isolate a null mutant lacking all CRWN genes (Wang *et al.*, 2013). These results clearly indicate that CRWNs are indispensable for cell viability, similarly to lamins being essential in vertebrate cells.

The novel nuclear envelope protein KAKU4

In a genetic screen for mutants having abnormal nuclear morphology, the *Arabidopsis kaku4* mutant was isolated (Goto *et al.*, 2014). Compared to wild-type nuclei, nuclei in *kaku4* are more spherical and smaller, similar to those in *crwn1* and *crwn4*. KAKU4 is a protein with no discernible functional domain and is localised at the inner nuclear

membrane. KAKU4 has an ability to modulate nuclear membrane morphology in a dose dependent manner, as seen for CRWN1. Moreover, this KAKU4-induced nuclear membrane alteration is dramatically enhanced in the presence of high levels of CRWN1. Immunoprecipitation and yeast two-hybrid assays revealed that KAKU4 physically interacts with CRWN1 and CRWN4, indicating that KAKU4-CRWN complexes are plant specific components in the lamina-like structure. The *kaku4 crwn1* double mutant exhibits neither synergistic nor additive effects on nuclear morphology compared with single mutants, supporting the notion that KAKU4 and CRWN1 work together in the same pathway.

In contrast to CRWNs, which are widely conserved in land plants (Ciska and Moreno Diaz de la Espina, 2013) and essential for viability (Wang *et al.*, 2013), KAKU4 appears to be present only in seed plants (Goto *et al.*, 2014) and to be non-essential. These results suggest that plants might have acquired KAKU4 as a non-essential lamina component to support CRWN function during their evolution. Animal lamins are known to bind many INM proteins *in vitro* and *in vivo* (Gruenbaum *et al.*, 2005). Lamin-binding proteins may act as adaptors necessary for the lamina structure to regulate large-scale chromatin organisation, the spacing of NPCs, positioning of the nucleus in the cell, and the reassembly of the NE after mitosis. To further understand the plant lamina-like structure, other lamina-associated proteins that surround the nuclear periphery need to be identified. It is assumed that CRWNs and KAKU4 are the best baits for isolating components of the plant lamina-like structure.

Linker of nucleoskeleton and cytoskeleton (LINC) complex

Recent findings demonstrate that the nuclear lamina is connected with cytoskeletal elements through the linker of nucleoskeleton and cytoskeleton (LINC) complex. The LINC complexes span the double membrane of the NE and mechanically couples the

nucleoskeleton and cytoskeleton (Simon and Wilson, 2011). This physical linkage affects force-induced changes in nuclear morphology and gene expression, known as mechanotransduction (Wang *et al.*, 2009). In mammals, the molecular units of LINC complexes are KASH (Klarsicht, ANC-1 and SYNE homology) domain-containing nesprins (NE spectrin-repeat proteins) and SUN (Sad1 and UNC-84) domain-containing proteins. KASH proteins are localisd on the ONM, while SUN proteins are localised on the INM and interact with the lamina structure (Mejat and Misteli, 2010; Simon and Wilson, 2011; Zhou and Meier, 2013). LINC complexes regulate various nuclear processes, including nuclear morphology, positioning, migration, and chromosomal organization. In human, mutations in LINC components lead to diseases by affecting cellular organization and function not just in the nucleus but throughout the cell (Mejat and Misteli, 2010). This indicates that LINC complexes play an essential role in cellular activities.

Plant SUN and KASH proteins

SUN domain proteins are evolutionarily conserved NE proteins. In animals, the nucleoplasmic domains of SUN proteins interact with several nuclear proteins, including lamins (Burke and Stewart, 2013). *Arabidopsis* has five SUN proteins, which can be divided into two subgroups, namely classical Cter-SUN (SUN1 and 2) and mid-SUN (SUN3, 4, and 5) (Graumann *et al.*, 2010; Graumann *et al.*, 2014; Murphy *et al.*, 2010; Oda and Fukuda, 2011; Tatout *et al.*, 2014). Cter-SUNs are composed of a transmembrane domain followed by a C-terminal SUN domain, while mid-SUNs are composed of a transmembrane domain followed by the SUN domain and two transmembrane domains. Fluorescent protein fusion experiments clearly demonstrated that Cter-SUNs (SUN1 and 2) are preferentially localised on the NE with low mobility and form homomers and heteromers dependent on their coiled-coil domains (Graumann *et al.*, 2010). In contrast, mid-SUNs (SUN3 and SUN4)

accumulate at the NE and endoplasmic reticulum, suggesting that they have different roles from Cter-SUNs. Loss-of-function mutations in two *Cter-SUN* genes (*SUN1* and *2*) results in spherical shaped nuclei in epidermal cells of various tissues, compared to characteristic spindle shaped nuclei in the wild type (Oda and Fukuda, 2011; Zhou *et al.*, 2012). Recently, *Arabidopsis* knockout mutants of mid-SUNs have also been investigated. *sun3* mutant has more spherical nuclei than the wild type, while nuclei in the *sun4 sun5* double mutant show smaller but normal spindle shaped nuclei. Although single and double mutants of mid-SUN show no obvious growth defects, triple mid-sun mutation leads to lethality (Graumann *et al.*, 2014). It is, therefore, suggested that mid-SUNs have redundant functions for cellular viability. Taken together, these results suggest that both Cter-SUN and mid-SUN play independent roles in nuclear morphology in plants, possibly because they interact with different KASH-domain proteins.

All known integral membrane proteins that are specifically localised on the ONM are thought to be KASH proteins (Starr and Fridolfsson, 2010). Most animal KASH proteins are structurally characterized by a C-terminal transmembrane domain and a C-terminal tail of fewer than 35 residues that is terminated by a PPPX motif, which is essential for SUN protein interaction (Starr and Fridolfsson, 2010; Wilhelmsen *et al.*, 2006). The first identified KASH proteins in plants are WIPs (WPP domain-interacting proteins), which have a C-terminal VVPT motif for interaction with SUN proteins (Zhou *et al.*, 2012). WIPs were also found to interact with other ONM proteins, WITs (WPP domain-interacting tail-anchored proteins) (Zhao *et al.*, 2008). Knockout mutants of either WIPs or WITs exhibit abnormal nuclear morphology in root cells, similar to Cter-SUN loss-of-function mutants (Tamura *et al.*, 2013; Zhou and Meier, 2014; Zhou *et al.*, 2012), indicating functional linkage of the plant SUN-KASH system on the NE.

A membrane-based yeast two-hybrid screen with Cter-SUNs as baits identified another plant KASH protein, TIK (TIR-KASH protein) (Graumann *et al.*,

2014). TIK contains a putative Toll-Interleukin-Resistance (TIR) domain (Mitcham *et al.*, 1996) followed by a transmembrane domain and a PPPS motif, which is a characteristic signature of the KASH domain. TIK proteins are conserved in only a few *Brassicaceae* species, although TIR-domain proteins are conserved in many species. This suggests that TIKs arose during evolution to function as unique KASH proteins linked to species-specific processes. In *Arabidopsis tik* mutant, nuclei show reduced width and length as well as smaller surface area. This indicates that TIK, like other plant LINC complex components, plays a key role in nuclear morphology, specifically nuclear size. Recently, to identify putative KASH domain proteins in genome databases, a Java-based DORY program has been developed (Zhou and Meier, 2014). *In-silico* screening with this program successfully identified five plant proteins (SINE1-5), which were experimentally verified to localise on the NE and to interact with Cter-SUN (*Arabidopsis* SUN1 and SUN2). However, the involvement of the SINE proteins in nuclear morphology remains unknown.

Myosin XI-i, a member of the plant-specific myosin class XI

In animal cells, the microtubule motors kinesin and dynein are known to interact with KASH proteins to regulate nuclear migration in various cellular processes (Gundersen and Worman, 2013). In contrast, in plant cells, pharmacological studies have indicated that nuclear migration depends solely on the actin system and not microtubules (Chytilova *et al.*, 2000). This suggests that the mechanisms of nuclear migration are totally distinct in plant and animal cells. A forward genetic screen of *Arabidopsis* for changes of nuclear morphology revealed that myosin XI-i (KAKU1) regulates nuclear morphology and migration (Tamura *et al.*, 2013). In *myosin xi-i*/*kaku1* mutant, the NE is abnormally invaginated, resulting in smaller and more spherical nuclei. Nuclear movement in root cells of the mutant is significantly impaired. *Myosin XI-i* encodes a plant specific myosin, which is widely conserved in land plants

and is localised on the NE. Biochemical and genetic analyses indicate that myosin XI-i specifically interacts with the SUN-WIP-WIT complex. It was also found that WIT proteins on the ONM are required for anchoring myosin XI-i to the NE, indicating that myosin XI-i functions as a molecular linker between plant LINC complexes and the actin cytoskeleton. Plants might have evolved this unique machinery involving actin and a myosin motor system to enable long-distance nuclear movement in cells.

Microtubules and γ-TUBULIN COMPLEX PROTEIN3-INTERACTING PROTEINs (*GIPs*)

Several reports demonstrate that microtubules are intimately involved with nuclear morphology. Treatment with aphidicolin, an inhibitor of DNA polymerase α, increases nuclear DNA content and elongation of nuclei in tobacco cultured cells (Yasuhara and Kitamoto, 2014). This elongation was found to require both intact microtubules and actin filaments. During the elongation, microtubule bundles are longitudinally arranged and associate with the nuclear surface. In contrast, actin filaments are suggested to serve as scaffolding in the formation of the microtubule alignment associated with the elongating nucleus. These results suggest that microtubules preferentially regulate nuclear morphology in certain conditions. Aphidicolin-treated tobacco cells are a useful tool for investigating the relationships between nuclear morphology and microtubule bundles.

The GIPs are small γ-tubulin complex components and are widely conserved in eukaryotes (Batzenschlager *et al.*, 2014; Batzenschlager *et al.*, 2013; Janski *et al.*, 2012). In *Arabidopsis*, GIP1 and GIP2 are localised on the NE during interphase (Batzenschlager *et al.*, 2013), as well as on microtubule mitotic arrays in dividing cells (Janski *et al.*, 2012). The *gip1 gip2* double mutant exhibits microtubule disruption and abnormal spindle polarity, leading to severe growth defects and sterility (Janski *et al.*, 2012). These results indicate that GIP proteins play a key role in microtubule

organization through the recruitment of γ-tubulin complexes at the NE. Importantly, the *gip1 gip2* mutant also shows deformed nuclei and abnormal distribution of NPCs and SUN1, indicating that GIPs are important for establishing NE functional rigidity. A yeast two hybrid screen revealed that GIP1 interacts with a short cytosolic C-terminal domain of AtTSA1, which is a type I membrane protein localised at the nuclear periphery (Batzenschlager *et al.*, 2013) and the cytoplasmic vesicles (Suzuki *et al.*, 2005). These results provide important evidence for the involvement of a γ-tubulin complex component in both nuclear morphology and NE organization.

Nucleoporins

The nuclear pore complex (NPC) is one of the largest protein complexes in cells and comprises more than 30 different proteins called nucleoporins (Nups) (Cronshaw *et al.*, 2002; Grossman *et al.*, 2012; Rout *et al.*, 2000). Structurally, the NPC is divided into three ring-like structures, the nuclear ring, the central spoke ring, and the cytoplasmic ring (Grossman *et al.*, 2012). Electron microscopy studies have revealed that the general morphology of the NPC is conserved among eukaryotes (Callan and Tomlin, 1950; Roberts and Northcote, 1970; Yoo and Bayley, 1967). Accordingly, in contrast to other NE proteins, NPC components are evolutionarily conserved between yeast (Rout *et al.*, 2000), animals (Cronshaw *et al.*, 2002), and plants (Ohtsu *et al.*, 2014; Tamura *et al.*, 2010).

There is ample evidence that some nucleoporins have both redundant and specific functions in plants (Parry, 2013). *Arabidopsis* Nup136/Nup1, which is a component of the NPC nuclear ring, has the ability to change nuclear morphology. Knockout of *Nup136/Nup1* results in nuclei changing from a spindle to a spherical morphology (Tamura *et al.*, 2010), while over-expression of Nup136/Nup1 results in super-elongated nuclei (Tamura and Hara-Nishimura, 2011). This result suggests that the level of accumulation of Nup136 in the cell is critical for regulating nuclear

morphology in plants. The vertebrate Nup153, which is a functional homolog of Nup136/Nup1, is the only nucleoporin that interacts directly with lamin proteins (Al-Haboubi *et al.*, 2011; Smythe *et al.*, 2000). Zhou *et al*. reported that Nup153 depletion induces alteration of nuclear lamina organisation in HeLa cells, resulting in abnormal NE structure (Zhou and Pante, 2010). Interestingly, with a feSEM, Fiserova *et al*. revealed that plant NPCs are interconnected by filamentous lamina-like structures in tobacco cells (Fiserova *et al.*, 2009). Based on these results, Nup136/Nup1 has been proposed to interact with plant lamina-like structures to maintain nuclear morphology.

Nuclear morphology has also been investigated in several other *Arabidopsis* nucleoporin mutants, including *nup54*, *nup58*, *nup62*, *nup85*, *seh1*, and *nup160* (Parry, 2014). Among these, only *nup160* exhibits significantly more spherical nuclei in root cells. In *N. benthamiana* plants, RNA silencing of *Nup88* leads to irregular nuclear morphology of leaf cells (Ohtsu *et al.*, 2014). As in the case of Nup136/Nup1, it is also possible that Nup160 and Nup88 indirectly interact with the lamina-like structure. Alternatively, the nucleoporins in the NPC may transport specific cargo proteins that play important roles in maintaining nuclear morphology. It was reported that nuclear import rates correlate with nuclear size and two transport factors (importin alpha and Ntf2) modulate lamin B3 import in *Xenopus* (Levy and Heald, 2010). It is therefore assumed that transport mechanisms, through the interaction of certain nucleoporins with cargos, are physiological regulators of nuclear morphology.

Conclusions

For a long time, the plant NE was an enigmatic structure, mainly because of a lack of easily identifiable homologues among its constituents. This view of the plant NE has changed in recent years with the discovery of its components and dynamic structure. In this review, we attempted to provide an overview of the plant NE proteins involved

in nuclear morphology with the aim of highlighting how rapidly our view of the NE structure is changing. A fascinating question that emerges from these recent discoveries is how changes in nuclear morphology affect essential nuclear processes. For example, although evidence for a plant LINC complex connecting the nucleus to the cytoskeleton is growing, its interaction with chromatin is still unknown. Dissecting the regulation and functions of the linkage between the NE and nuclear contents is an exciting and important challenge for the future.

Discovery of additional plant NE interacting proteins is also needed to clarify the constituent NE components. To achieve this, a combination of various approaches, including proteomics, forward genetics, *in-silico* screening, and interactomics, is necessary, because these methods do not depend on analogy between plants and other organisms. In the near future, we anticipate that these sophisticated experimental approaches will begin to resolve many of the questions and inconsistencies that still bedevil studies of the plant NE.

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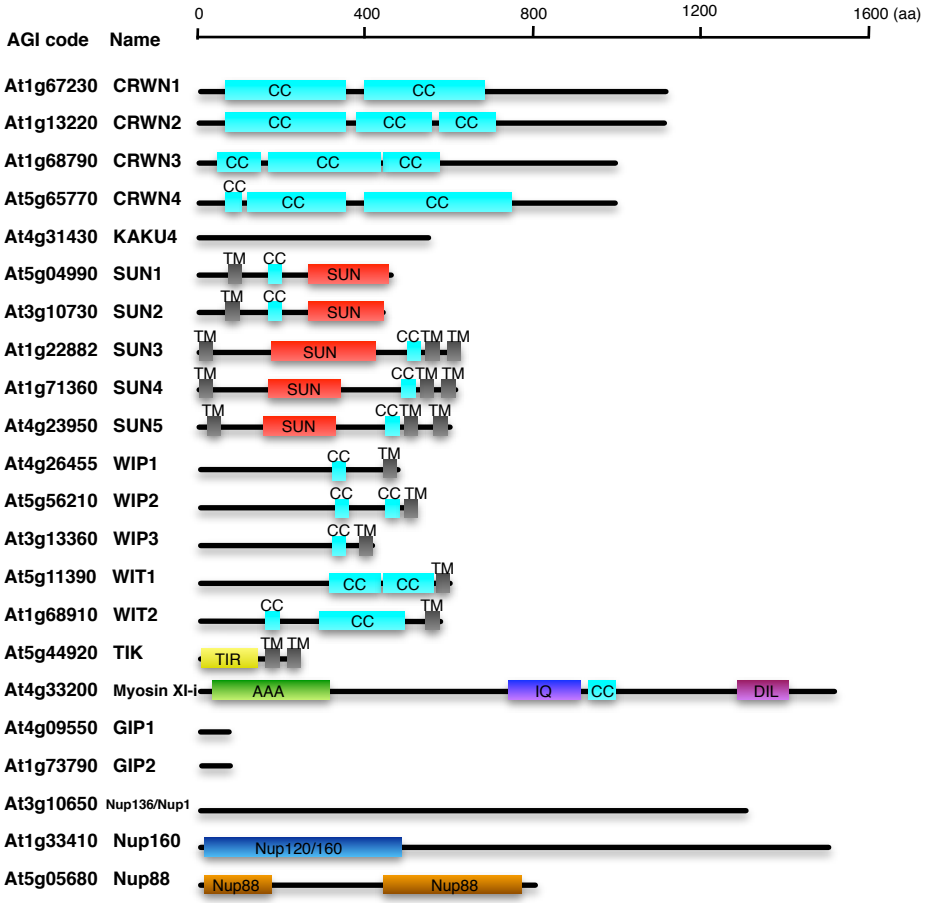
Table 1. Summary of the plant nuclear envelope proteins involved in nuclear morphology

Protein	AGI code and accession number	Reference
Lamina-like structure		
CRWN	CRWN1 (At1g67230), CRWN2 (At1g13220), CRWN3 (At1g68790), CRWN4 (At5g65770)	Dittmer *et al*. (2007), Goto et al. (2014), Sakamoto and Takagi (2013), Wang *et al*. (2014)
KAKU4	At4g31430	Goto et al. (2014)
LINC complex and cytoskeleton		
C-terSUN	SUN1 (At5g04990), SUN2 (At3g10730)	Graumann *et al*. (2010), Oda and Fukuda (2011), Zhou *et al*. (2012)
mid-SUN	SUN3 (At1g22882), SUN4 (At1g71360), SUN5 (At4g23950)	Graumann *et al*. (2014)
WIP	WIP1 (At4g26455), WIP2 (At5g56210), WIP3 (At3g13360)	Zhou *et al*. (2012), Zhou and Meier (2014)
WIT	WIT1 (At5g11390), WIT2 (At1g68910)	Zhao *et al*. (2008), Zhou and Meier (2014)
TIK	At5g44920	Graumann *et al*. (2014)
Myosin XI-i	At4g33200	Tamura et al. (2013)
GIP	GIP1 (At4g09550), GIP2 (At1g73790)	Batzenschlager *et al*. (2014), Batzenschlager *et al*. (2013), Janski *et al*. (2012)
Nuclepporins		
Nup136/Nup1	At3g10650	Tamura *et al*. (2010), Tamura *et al*. (2011)
Nup160	At1g33410	Parry (2014), Tamura *et al*. (2010)
Nup88	*Arabidopsis* (At5g05680), Tobacco (AB897508 and AB897509)	Ohtsu *et al*. (2014), Tamura *et al*. (2011)

Figure legends

Fig 1. Schematic representation of the predicted domain features of plant nuclear envelope proteins in this review. Domain architectures were predicted using domain analysis tools on the website (InterPro: http://www.ebi.ac.uk/interpro/, SOSUI: http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html/, SMART: http://smart.embl-heidelberg.de/). AAA, ATPase associated with a variety of cellular activities; CC, coiled coil; DIL, dilute; IQ, IQ domain, Nup, nucleoporin: SUN, Sad-1/UNC-84; TIR, Toll/Interleukin-1 receptor homology; TM, transmembrane.



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